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The Satiating Effect of Dietary Protein Is Unrelated to Postprandial Ghrelin Secretion

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Context: Increasing dietary protein relative to carbohydrate and fat enhances weight loss, at least in part by increasing satiety. The mechanism for this is unclear.

Objective: The objective of this study was to compare the effects of isocaloric test meals with differing protein to fat ratios on fasting and postprandial ghrelin, insulin, glucose, appetite, and energy expenditure before and after weight loss on the respective dietary patterns.

Design: The study design was a randomized parallel design of 12 wk of weight loss (6 MJ/d) and 4 wk of weight maintenance (7.3 MJ/d) with meals administered at wk 0 and 16.

Setting: The study was performed at an out-patient research clinic.

Patients and Other Participants: Fifty-seven overweight (body mass index, 33.8 ± 3.5 kg/m^2) hyperinsulinemic men (n = 25) and women (n = 32) were studied.

Interventions: High-protein/low-fat (34% protein/29% fat) or standard protein/high-fat (18% protein/45% fat) diets/meals were given.

Main Outcome Measures: The main outcome measures were weight loss and fasting and postprandial ghrelin, insulin, glucose, appetite, and energy expenditure before and after weight loss.

Results: Weight loss (9.2 ± 0.7 kg) and improvements in fasting and postprandial insulin and glucose occurred independently of diet composition. At wk 0 and 16, subjects wanted less to eat after the high-protein/low-fat than the standard protein/high-fat meal ($P = 0.02$). Fasting ghrelin increased (157.5 ± 3.4 pg/ml or 46.6 ± 1.0 pmol/liter; $P < 0.001$), and the postprandial ghrelin response improved with weight loss ($P = 0.043$) independently of diet composition. Postprandial hunger decreased with weight loss ($P = 0.018$) and was predicted by changes in fasting and postprandial ghrelin ($r^2 = 0.246$; $P = 0.004$). Lean mass was the best predictor of fasting ($r^2 = 0.182$; $P = 0.003$) and postprandial ghrelin ($r^2 = 0.096$; $P = 0.039$) levels.

Conclusions: Exchanging protein for fat produced similar weight loss and improvements in metabolic parameters and ghrelin homeostasis. The reduced appetite observed with increased dietary protein appears not to be mediated by ghrelin homeostasis. **(*J Clin Endocrinol Metab* 90: 5205–5211, 2005)**

FOR OVERWEIGHT AND obese men and women, particularly those with visceral obesity, weight loss decreases the risks of cardiovascular disease and type II diabetes mellitus. The conventional approach for achieving and maintaining weight loss is a high-carbohydrate, low-fat diet (1). However, long-term compliance with this dietary pattern is poor (2), and a potential worsening of the metabolic profile may occur (3). Alternative macronutrient profiles may have more favorable effects on the metabolic profile and may also optimize weight loss in an *ad libitum* setting. Approaches now being examined include lower carbohydrate dietary patterns, higher in either protein or monounsaturated fat. In both isocaloric energy-restricted and *ad libitum* feeding environments, these approaches can reduce weight (4–6), waist circumference (6), and total (4–6) and abdominal fat mass (7) and maintain lean mass (3). Increased dietary protein may facilitate weight loss through its greater satiating (8) and thermic effects (9, 10). Accordingly, replacing a moderate amount of fat and carbohydrate for protein may be a reasonable dietary option to optimize weight loss and may maximize metabolic improvements and enhance the maintenance of weight loss.

The differential satiating effects of altering macronutrient composition may be mediated by gastrointestinal hormones, including the orexigenic stomach-derived peptide, ghrelin. Ghrelin stimulates GH secretion through its action as an endogenous ligand for the hypothalamic-pituitary GH secretagogue receptor. In addition, ghrelin is implicated in long-term energy homeostasis and acute meal initiation. Ghrelin levels increase preprandially, decrease postprandially (11), and stimulate hunger and food intake (12) through action on the hypothalamic arcuate nucleus. Total and active fasting ghrelin levels are decreased in human obesity (13), which may represent a compensatory response to a sustained positive energy balance (11). For obese individuals, it is proposed that postprandial ghrelin suppression may be partially or fully impaired (14–16). Suppression of postprandial hunger may therefore be diminished, which could lead to earlier reinitiation of feeding signals and, consequently, resistance to weight loss (15). This is consistent with delayed satiation documented in overweight individuals (17). The role, if any,

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Abbreviations: AUC, Area under the curve; BMI, body mass index; HOMA, homeostasis model assessment; HP-LF, high-protein, low-fat diet; MTT, meal tolerance test; REE, resting energy expenditure; RQ, respiratory quotient; SP-HF, standard protein, high-fat diet; TEF, thermic effect of feeding; VAS, visual analog scale.

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of macronutrient composition in the regulation of ghrelin remains unclear. Increasing dietary carbohydrate acutely (18) or chronically in weight-maintaining diets (19) may suppress postprandial ghrelin more than increasing dietary fat, although data are conflicting (15). Ghrelin may also induce a positive energy balance by reducing energy expenditure (20). The relationship among ghrelin, satiety, and energy expenditure and its potential implications for the thermic and satiating effects of dietary protein are thus unclear.

The objective of this study was to compare the short-term effects of two isocaloric, energy-restricted, carbohydrate-matched diets and test meals containing either increased protein, low-fat (HP) or standard protein, high-fat diet (SP; monounsaturated fat-enriched) on weight loss and fasting and postprandial ghrelin levels. In addition, the relationship between ghrelin homeostasis and changes in body weight and composition, appetite, energy expenditure, and fasting and postprandial metabolic variables were examined.

Subjects and Methods

Subjects

Overweight hyperinsulinemic men and women (n = 73), aged 20–65 yr, with fasting serum insulin levels greater than 15 mU/liter and a body mass index (BMI) of 27–40 kg/m^2 were recruited by public advertisement. Subjects were excluded if diabetes mellitus, microalbuminuria, or a history of liver, unstable cardiovascular, respiratory, gastrointestinal disease, malignancy, pregnancy. or lactation was present. Premenopausal women or claustrophobic subjects were excluded from participating in the measurement of energy expenditure. All experimental procedures were approved by the human ethics committees of the Commonwealth Scientific Industrial Research Organization and the Royal Adelaide Hospital, and all subjects provided written informed consent.

Dietary protocol

The prescribed diets were: 1) high-protein, low-fat diet [HP-LF; 40% energy as protein (136 g/d), 30% fat (46 g/d; <10% saturated fat), 30% carbohydrate, and 21 g fiber]; and 2) standard protein, high-fat diet [SP-HF; 20% energy as protein (67 g/d), 50% fat (76 g/d; <10% saturated fat), 30% carbohydrate, and 27 g fiber]. Most subjects were sedentary at baseline and were asked to continue their usual physical activity levels and to refrain from drinking more than two standard glasses of alcohol per week throughout the study. Subjects followed fixed meal plans and were supplied with key foods making up 60% of their energy intake (21). Subjects met fortnightly with a dietitian for education on quantification and recording of their daily food intake on daily checklists and to modify the dietary regimen based on compliance and weight loss. Nutritional intake was assessed from fortnightly 3-d consecutive food records (one weekend day and two weekdays) with the use of Diet 1/Nutrient Calculation software (Xyris Software, Highgate Hill, Australia). Dietary compliance was determined by subject adherence to the macronutrient profiles and assessment of serum creatinine and 24-h urinary urea to creatinine ratio at wk 0 and 16 (21).

Experimental protocol

The study was conducted over 16 wk on an out-patient basis. Subjects were matched for fasting serum insulin, BMI, age, and gender. The two groups were then randomly assigned to either the HP-LF or SP-HF diet. Both dietary groups underwent 12 wk of energy restriction (6081 kJ/d), followed by 4 wk (7346 kJ/d) at energy balance on the same macronutrient composition.

Each month, subjects attended the out-patient clinic on 2 consecutive days. At wk 0, 12, and 16 subjects, after having fasted overnight, were weighed in light clothes with no shoes (Mettler scales, model AMZ14; A&D Mercury, Kinomoto, Japan). At wk 0 and 16, total fat mass, total lean mass, and abdominal fat mass were assessed by whole-body, dual-energy x-ray absorptiometry (densitometer XR36; Norland Medical Systems, Fort Atkinson, WI; coefficient of variation, 2.3 ± 0.7% for total body fat mass and 2.1 ± 0.4% for lean mass).

At wk 0 and 16, total energy expenditure was measured for 30 of the subjects (18 men and two women). In all subjects, a 3-h meal tolerance test (MTT) was performed with a test meal representative of the allocated diet; HP-LF (2636 kJ; 37% of energy as protein, 30% fat, and 32% carbohydrate) or SP-HF (2586 kJ; 18% of energy as protein, 49% fat, and 32% carbohydrate).

Appetite sensations and venous insulin, glucose, and ghrelin concentrations were measured before consuming the meal and 30, 60, 120, and 180 min after the test meal. Fasting resting energy expenditure (REE) and respiratory quotient (RQ) were measured by indirect calorimetry (Deltatract metabolic monitor, Datex Division Instrumentarium Corp., Helsinki, Finland) (21). After consumption of the test meal, RQ and REE values were recorded every 20 min for 180 min, adjusted from fasting values, and averaged to determine postprandial RQ and thermic effect of feeding (TEF) (21).

Subjective hunger, fullness, satiety, and desire to eat were assessed using a validated 100-mm linear visual analog scale (VAS) as previously described (21). Subjects were asked to make a single vertical mark on each scale between the extremes (*e.g.* hungry to not hungry) to indicate their feelings at that time. The change in ratings from baseline was quantified. Subjects did not discuss their ratings with each other and could not refer to their previous ratings when marking the VAS. Total (glucose and insulin), incremental (ghrelin), and net (visual analog scores) areas under the curve (AUC) during the 3-h MTT were calculated geometrically using the trapezoidal rule (22).

Serum insulin, plasma glucose, and urinary urea and creatinine were measured as previously described (21). Plasma ghrelin (total) was measured using a commercially available RIA (CV, <4.5%; Phoenix Pharmaceuticals, Inc., Belmont, CA). The homeostasis model assessment (HOMA) was used as a surrogate measure of insulin sensitivity [fasting insulin (mU/liter) × fasting glucose (mmol/liter)/22.5] (23).

Statistical analyses

The characteristics of the subjects are presented as the mean ± SEM, except where indicated. Results are presented for 57 subjects, except fasting glucose, insulin, HOMA, and dual-energy x-ray absorptiometry (n = 56); AUC glucose and insulin (n = 54); fasting ghrelin (n = 47); AUC ghrelin (n = 45); and VAS (n = 50) due to incomplete data. Two-tailed statistical analysis was performed using SPSS for Windows 10.0 software (SPSS, Inc., Chicago, IL) with statistical significance set at an α level of $P < 0.05$. Baseline measurements were assessed using two-factor ANOVA, with diet and gender as fixed factors. Comparisons between time points were assessed using repeated measures ANOVA, with diet and gender as between-subject factors. In specific analyses, baseline weight and glucose were included as covariates. Week 0 and 16 response curves after the test meals were compared using a four-way, repeated measures ANOVA, with week and blood sampling time as within-subject factors and diet and gender as between-subject factors. Where an interaction was observed, *post hoc* pairwise comparisons were performed. Relationships between variables were examined using bivariate and partial correlations, analysis of covariance, and multiple linear regression.

Results

Subjects

Fifty-seven (25 men and 32 women; mean age, 50.3 ± 9.9 yr; mean BMI, 34.0 ± 3.5 kg/m^2; mean weight, 97.2 ± 14.0 kg; mean ± SD) completed the intervention. Sixteen subjects dropped out of the study (work commitments, n = 2; health reasons, n = 3; personal reasons, n = 6; lost to follow-up, n = 5), five subjects before study commencement, and 11 subjects during the study. There were no differences in the characteristics of subjects in each diet group at baseline (Table 1).

Baseline weight (111.6 ± 3.8 *vs.* 90.0 ± 2.4 kg; $P < 0.001$) and fasting glucose (107.1 ± 7.1 *vs.* 94.6 ± 1.8 mg/dl or 6.0 ± 0.4 *vs.* 5.3 ± 0.1 mmol/liter; $P = 0.048$) were higher for males

TABLE 1. Subject characteristics at baseline

	SP-HF diet		HP-LF diet	
	Male (n = 13)	Female (n = 17)	Male (n = 12)	Female (n = 15)
Age (yr)	49.9 ± 11.4	48.0 ± 11.0	50.3 ± 0.8	53.3 ± 8.2
Weight (kg)[a]	111.6 ± 13.6	90.0 ± 10.1	100.3 ± 10.4	90.5 ± 11.0
BMI (kg/m^2)	34.8 ± 3.7	34.4 ± 3.5	32.2 ± 3.2	34.4 ± 3.3
Glucose (mg/dl)[b]	112.3 ± 43.6	94.0 ± 11.8	101.4 ± 13.1	94.7 ± 6.4
Insulin (mU/liter)	12.2 ± 7.5	12.2 ± 6.4	12.4 ± 6.1	14.0 ± 7.6
HOMA	3.4 ± 2.4	2.9 ± 1.6	3.1 ± 1.5	3.3 ± 1.7

Data are expressed as means ± SD. Measurements were made at the wk-0 visit and were assessed using two-way ANOVA with diet and gender as the fixed factors. For conversion from mg/dl to mmol/liter for glucose, multiply by 0.056. For conversion from mU/liter to pmol/liter for insulin, multiply by 6.95. For insulin/glucose/HOMA results: SP-HF (male n = 13, female n = 16); HP-LF (male n = 12, female = 15).

[a] Men were significantly different from women, $P < 0.001$.

[b] Men were significantly different from women, $P = 0.048$.

than females. Both diets were well tolerated, with no adverse events reported, and all subjects complied with the dietary intervention based on the urinary urea/creatinine ratio and reported individual macronutrient profiles (21). The percentage of energy derived from the macronutrients of both diets remained the same during energy balance as during the energy-restricted phase, but the energy content of each diet increased to achieve energy balance ($P < 0.001$). Total energy intake was not different between diet during energy restriction or energy balance (21).

Body weight, body composition, and fasting and postprandial insulin and glucose

As previously reported (21), a mean weight loss of 9.2 ± 0.7 kg or 9.5% ($P < 0.001$) occurred independently of diet, and weight was maintained effectively over the 4-wk energy balance stage ($P = 0.07$). From wk 0–16, reductions in fat mass (13.9 ± 1.5%; $P < 0.001$), abdominal fat mass (17.1 ± 2.0%; $P < 0.001$), lean mass (6.0 ± 0.6%; $P < 0.001$), fasting glucose (3.5 ± 1.5%; $P = 0.024$), fasting insulin (13.8 ± 9.3%; $P = 0.001$), and fasting HOMA (13.3 ± 11.7%; $P = 0.001$) occurred with no diet or diet by gender interactions (Table 2 and Fig. 1, A and B). There was a time by gender interaction, such that the men lost more weight and abdominal fat than the women [respectively, 10.9 ± 1.2 *vs.* 7.9 ± 0.8 kg ($P = 0.028$) and 2.1 ± 0.3 *vs.* 1.3 ± 0.3 kg ($P = 0.039$); Table 2]. At wk 16 compared with wk 0, there were reductions in the postprandial glucose (4.1 ± 1.6%; $P = 0.01$) and insulin (12.3 ± 6.9%; $P < 0.001$) concentrations, with no effect of diet or any diet by gender interaction (Fig. 1, A and B).

Fasting and postprandial ghrelin

At baseline, there were no differences in fasting plasma ghrelin concentrations between the diet groups. Fasting ghrelin concentrations increased from 402.5 ± 40.0 to 557.9 ± 35.5 pg/ml (119.1 ± 11.7 to 165.2 ± 10.5 pmol/liter) from wk 0 to 16 ($P < 0.001$), with no effect of diet or gender. Ghrelin concentrations decreased during the MTT at both wk 0 and 16. There was a change in the postprandial ghrelin profile from wk 0–16 ($P = 0.043$), such that the maximal postprandial decrease in ghrelin occurred at 120 min at wk 0 and at 60 min during wk 16 ($P < 0.05$). The decrease in ghrelin over the first 60 min of the postprandial period was significantly greater at wk 16 than at wk 0 (−77.2 ± 19.8 *vs.* −13.6 ± 19.2 pg/ml or −22.9 ± 5.9 *vs.* −4.0 ± 5.7 pmol/liter, respectively; $P = 0.017$). There was also a trend for the postprandial nadir to be increased from wk 0 to 16 (from −70.5 ± 10.0 to −109.5 ± 20.7 pg/ml or −20.9 ± 3.0 to −32.4 ± 6.1 pmol/liter; $P = 0.06$). There was a trend for the postprandial ghrelin AUC to be improved from wk 0 to 16, such that a 5196.3 ± 2993.5 pg/ml (1538.2 ± 886.1 pmol/liter) greater reduction in AUC ghrelin concentrations occurred ($P = 0.087$; Table 2 and Fig. 1C). There were no overall diet or diet by gender

TABLE 2. Body weight and composition, fasting HOMA, and postprandial glucose, insulin, ghrelin at wk 0 and 16 for subjects in the SP-HF and HP-LF groups

	SP-HF diet (n = 30)	HP-LF diet (n = 27)
Weight (kg)[a,b,c]		
Week		
0	99.4 ± 2.9	94.9 ± 2.2
12	90.3 ± 2.9	86.3 ± 2.3
16	90.0 ± 3.0	85.9 ± 2.3
Total fat mass (kg)[a,b]		
Week		
0	40.0 ± 1.7	37.2 ± 1.7
16	34.8 ± 1.9	32.3 ± 1.9
Abdominal fat (kg)[b,c,d]		
Week		
0	10.8 ± 0.4	9.5 ± 0.4
16	9.0 ± 0.5	7.9 ± 0.5
Total lean mass (kg)[a,b]		
Week		
0	55.7 ± 2.5	53.6 ± 2.4
16	52.2 ± 2.5	50.6 ± 2.2
HOMA[a]		
Week		
0	3.1 ± 0.4	3.2 ± 0.3
16	2.5 ± 0.3	2.1 ± 0.2
AUC glucose (mg/dl·180 min)[a,b,c]		
Week		
0	19,830.1 ± 1,108.6	18,783.6 ± 356.2
16	18,369.2 ± 496.7	18,057.7 ± 379.0
AUC insulin (mU/liter·180 min)[b]		
Week		
0	6,825.9 ± 637.7	7,842.6 ± 758.2
16	5,285.2 ± 502.8	5,950.7 ± 474.0
AUC ghrelin (pg/ml·180 min)		
Week		
0	8,420.7 ± 1,958.4	5,497.9 ± 1,606.8
16	16,962.2 ± 5,243.5	6,871.1 ± 1,823.0

Data are expressed as mean ± SEM. Data at wk 0 were assessed using two-way ANOVA with diet and gender as fixed factors. Data from wk 0 and 16 were assessed using repeated-measures ANOVA with time as within-subject factor and diet and gender as between-subject factors. For conversion from mg/dl to mmol/liter for glucose, multiply by 0.056. For conversion from mU/liter to pmol/liter for insulin, multiply by 6.95. For conversion from pg/ml to pmol/liter for ghrelin, multiply by 0.296. For HOMA/DEXA: SP-HF = 29, HP-LF n = 27; for AUC glucose/insulin SP-HF = 28, HP-LF n = 27; for AUC ghrelin SP-HF = 24, HP-LF n = 21.

[a] Significant difference between men and women at wk 0, $P < 0.05$.

[b] Effect of time from wk 0 to wk 16, $P < 0.05$.

[c] Effect of time*gender from wk 0 to wk 16, $P < 0.05$.

[d] Significant difference between diets at wk 0, $P < 0.05$.

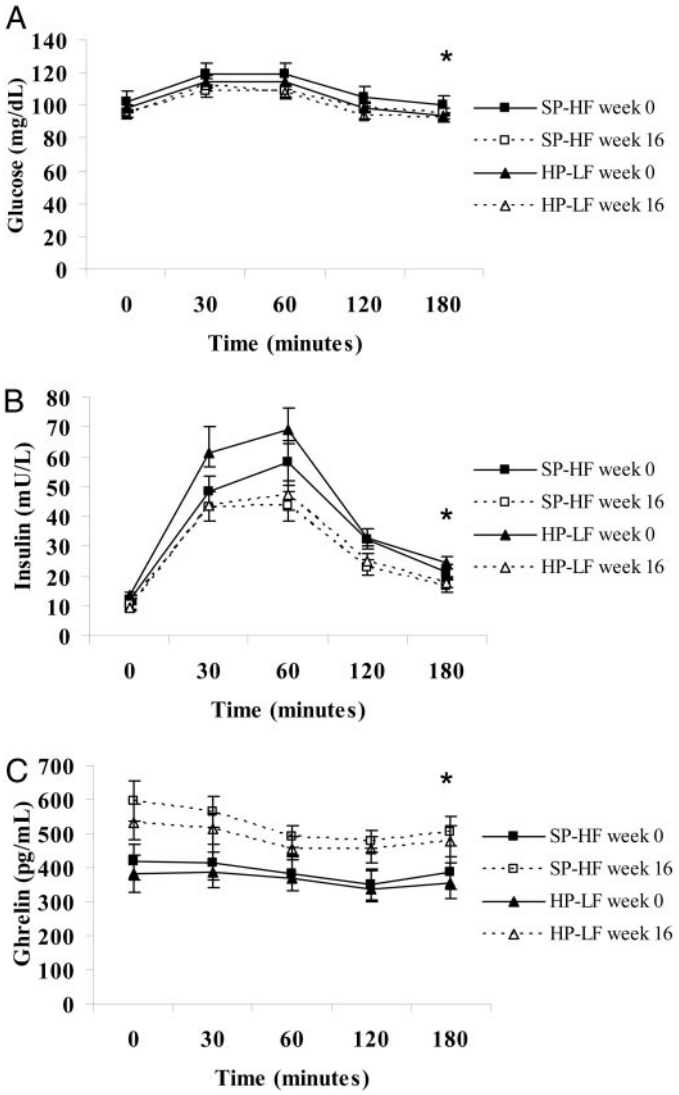

FIG. 1. Mean (±SEM) plasma glucose (A), insulin (B), and ghrelin (C) concentrations at baseline and 30, 60, 120, and 180 min after the ingestion of an SP-HF (■; n = 28 for glucose and insulin; n = 24 for ghrelin) or HP-LF (▲; n = 26 for glucose and insulin; n = 21 for ghrelin) test meal at wk 0 (*solid line*) and wk 16 (*dashed line*). Week 0 and 16 data were compared by repeated measures ANOVA, with week and blood sampling time as within-subject factors, and diet and gender as between-subject factors. For conversion from milligrams per deciliter to millimoles per liter for glucose, multiply by 0.056. For conversion from milliunits per liter to picomoles per liter for insulin, multiply by 6.95. For conversion from picograms per milliliter to picomoles per liter for ghrelin, multiply by 0.296. *, Significant effect of time from wk 0 to wk 16, $P < 0.01$.

effects on changes in postprandial ghrelin. Females had a higher fasting ghrelin at wk 0 and 16 ($P = 0.013$ and $P = 0.024$ for effect of gender) than males, but this effect was not significant when adjusted for baseline weight.

Energy expenditure and visual analog scores

In summary, from wk 0–16 postprandial RQ increased by 1.8% ($P = 0.007$), and there was a trend for REE to be reduced (4.0 ± 1.6%; $P = 0.055$), but neither was affected by diet composition. There was a time by diet effect ($P = 0.015$) for the TEF, such that it decreased by 3.6 ± 0.7% for the SP-HF diet compared with 0.32 ± 1% for the HP-LF diet (Table 3). There was a significant overall reduction in the 3 h hunger response ($P = 0.018$; Fig. 1A) and a significant increase in the fasting hunger scores (48.1 ± 4.2 *vs.* 35.7 ± 4.1 mm; $P = 0.026$) with no diet or diet by gender interaction. There was no diet or diet by gender effect on the desire to eat responses to the test meal; however, subjects wanted less to eat after the HP-LF compared with the SP-HF test meal at both wk 0 and 16 (overall diet effect, $P = 0.02$; Fig. 2C). For additional details on energy expenditure and visual analog score data, see the report by Luscombe-Marsh *et al.* (21).

Correlations and multiple regression analysis

Fasting ghrelin was correlated with weight and lean mass at both wk 0 ($r = -0.357$; $P = 0.014$ and $r = -0.427$; $P = 0.003$, respectively) and wk 16 ($r = -0.312$; $P = 0.033$ and $r = -0.355$; $P = 0.014$, respectively). The change in fasting ghrelin was correlated with the change in HOMA and AUC hunger ($r = -0.311$; $P = 0.033$ and $r = 0.346$; $P = 0.022$, respectively). Ghrelin AUC was correlated with lean mass at wk 0 ($r = -0.309$; $P = 0.039$). The change in ghrelin AUC was correlated with the change in HOMA ($r = -0.319$; $P = 0.033$). These relationships remained significant after adjustment for baseline weight.

The best predictor of fasting ghrelin was lean mass ($r^2 = 0.182$; $P = 0.003$). At wk 0, 25% of the variation in fasting ghrelin was explained by lean mass and fasting insulin. The best predictor of changes in fasting ghrelin with weight loss was a change in HOMA ($r^2 = 0.097$; $P = 0.033$). The best predictor of ghrelin AUC was lean mass ($r^2 = 0.096$; $P = 0.039$). The decrease in ghrelin AUC was primarily predicted

TABLE 3. Energy expenditure and respiratory quotient at wk 0 and 16 for subjects in the SP-HF and HP-LF groups

	SP-HF diet (n = 16)	HP-LF diet (n = 14)	Combined (n = 30)
REE (kJ/d)[a]			
Week			
0	8,961 ± 384	8,117 ± 298	8,567 ± 256
16	8,612 ± 390	7,775 ± 362	8,221 ± 274
TEF (%EI)[b,c]			
Week			
0	7.9 ± 0.56	7.2 ± 0.57	7.6 ± 0.40
16	4.3 ± 0.72	6.9 ± 0.95	5.5 ± 0.62
RQ[d]			
Week			
0	0.81 ± 0.013	0.82 ± 0.01	0.81 ± 0.00
16	0.80 ± 0.01	0.82 ± 0.009	0.81 ± 0.00
Av postprandial RQ[b]			
Week			
0	0.82 ± 0.006	0.82 ± 0.007	0.007 ± 0.007
16	0.83 ± 0.006	0.84 ± 0.006	0.018 ± 0.008

Data are expressed as mean ± SEM. TEF, Thermic response to a 2586 kJ SP, or 2636 kJ HP test meal expressed as the % increase per energy intake (EI) over 3 h; RQ, ratio of VCO_2/VO_2; postprandial RQ, average RQ over 3 h after the SP or HP test meal. Data from wk 0 and 16 data compared using repeated-measures ANOVA with time as the within-subject factor and diet and gender as between-subject factors.

[a] Effect of time from wk 0 to 16, $P = 0.055$.

[b] Effect of time from wk 0 to 16, $P < 0.02$.

[c] Significant time-by-diet interaction, $P < 0.02$.

[d] Significant time-by-diet-by-gender interaction, $P = 0.02$.

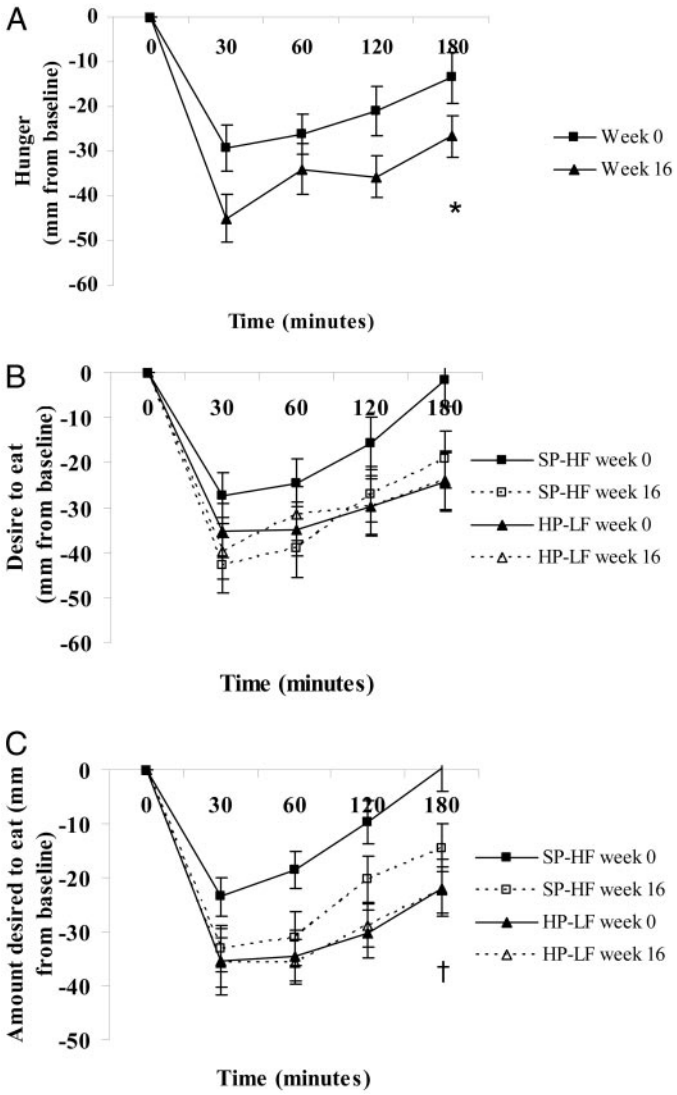

FIG. 2. Mean (±SEM) subjective VAS ratings for hunger (A), desire to eat (B), and the amount of food desired (C) at baseline and 30, 60, 120, and 180 min after the ingestion of an SP-HF (■; n = 27 for VAS) or HP-LF (▲; n = 23 for VAS) test meal at wk 0 (*solid line*) and 16 (*dashed line*). Week 0 and 16 data were compared by repeated measures ANOVA, with week and appetite rating time as within-subject factors, and diet and gender as between-subject factors. *, Significant effect of time from wk 0 to wk 16, $P < 0.01$. †, Significant overall effect of diet at both wk 0 and wk 16, $P = 0.02$.

by the increase in fasting ghrelin ($r^2 = 0.523$; $P < 0.001$). The change in hunger AUC after weight loss was significantly predicted by the change in fasting ghrelin ($r^2 = 0.117$; $P = 0.027$) and the change in fasting and ghrelin AUC ($r^2 = 0.246$; $P = 0.004$). There was no relationship between any measures of fasting or postprandial energy expenditure and fasting and postprandial ghrelin.

Discussion

Subjects wanted less to eat after a HP-LF compared with a SP-HF carbohydrate-equivalent test meal at wk 0 and 16, and there was an increased thermic effect after eating HP-LF compared with SP-HF test meals (21). These results are consistent with our previous findings (9, 10). There was no effect of altering the protein to fat ratio on fasting or postprandial ghrelin before or after weight loss, even though weight loss improved postprandial ghrelin regulation in association with improvements in postprandial hunger.

Despite our finding that the HP-LF test meals were more satiating than the SP-HF test meals, we observed no effect of isocaloric substitution of protein for fat on fasting or postprandial ghrelin. Previous findings examining the effect of varying dietary composition on ghrelin secretion are contradictory. Maximal suppression of postprandial ghrelin was reported for high carbohydrate compared with high fat isocaloric weight-maintaining diets (19) and meals (18). However, increases in postprandial ghrelin with high protein loads have been observed in a number of studies (15, 24). This would predict that appetite would be increased with dietary protein, whereas in reality the reverse occurs. In these studies, however, the energy content of the preloads were different for the test meals, separately shown to be related to the degree of postprandial ghrelin suppression (25). Additionally, our failure to observe a difference in postprandial ghrelin with varying macronutrient loads might be due to the preloads having similar effects on insulin and glucose responses. Changes in postprandial ghrelin with high carbohydrate preloads were significantly correlated with changes in postprandial insulin (26), suggesting a potential role of insulin in regulating postprandial ghrelin responses. However, other work has reported no relationship between postprandial ghrelin, insulin, and glucose (18). Furthermore, iv infusions of glucose have been found to either have no effect (27) or decrease postprandial ghrelin (28).

Consistent with the observations of other investigators (19, 29), we observed a postprandial decrease in plasma ghrelin levels in obese subjects, which was amplified after weight loss. The postprandial nadir occurred earlier at wk 16 compared with wk 0 (*i.e.* 60 min compared with 120 min), consistent with the timing of the postprandial decrease in lean individuals (30 min to 1 h) (14, 30). This confirms reports of improvements in postprandial ghrelin regulation with weight loss (16) and suggests that weight loss restores some measure of the normal regulatory role of ghrelin on hunger and meal initiation. It is unclear whether a particular amount of weight loss is required, because improvements in postprandial ghrelin have been reported after weight losses varying from 7.1–17.3 kg over 4–6 months (16, 29). Conversely, no improvement in postprandial ghrelin was observed with a weight loss of 3.8 kg over 3 months (19).

The increase in fasting hunger and decrease in postprandial hunger after weight loss were related to the change in fasting and postprandial ghrelin, as observed previously (16). The postprandial ghrelin improvements suggest a positive restoration of appetite control with weight loss. Previously, it has been proposed that the metabolic changes associated with weight loss contribute to weight regain through increasing hunger and decreasing satiety. There are, however, conflicting data on the effect of weight loss on fasting and postprandial appetite (31). It remains unclear how the observed changes in fasting or postprandial ghrelin levels reflect changes to long-term appetite regulation and thus maintenance of weight loss.

As previously shown (29), we observed an increase in fasting ghrelin with weight loss. Ghrelin may not be as critical in the control of acute feeding behavior as initially thought and may play a more important role in chronic energy balance. In support of this, an increase in circulating ghrelin was observed after aerobic exercise-induced weight loss without compensatory decreases in food intake (32). Markers of energy balance include markers of the size of adipose tissue stores, such as circulating adipocyte-derived factors or hormones such as leptin or insulin. We have confirmed findings that ghrelin correlates negatively with body mass (33). We also found that lean mass predicted fasting ghrelin levels and observed a negative relationship between ghrelin and lean body mass that has not previously been reported (34). Ghrelin is commonly associated with body fat (33), although ghrelin and the GH secretagogue receptor mRNA (type 1b) have been located in muscle in similar quantities as in adipose tissue (35). Ghrelin administration increases lean body mass in humans (36), and GH stimulates skeletal muscle protein synthesis (37). Our observed association may be reflective of a separate function of ghrelin on energy homeostasis, potentially related to its effects on stimulation of GH secretion.

The change in ghrelin was negatively correlated with and related to the change in surrogate markers of insulin sensitivity. This remained significant after adjustment for weight loss, suggesting a role of insulin in long-term regulation of ghrelin. Fasting ghrelin has previously been found to correlate negatively with fasting insulin (28, 33), and obese subjects who are insulin sensitive have higher fasting ghrelin than obese insulin-resistant subjects (38). Moreover, insulin resistance was reported to be a significant predictor of ghrelin concentrations independently of BMI (38). Obese and insulin-resistant populations, such as subjects with type 2 diabetes mellitus (39), Pima Indians (33), and polycystic ovary syndrome patients (16), all displayed reduced fasting ghrelin levels or blunted improvements in fasting or postprandial ghrelin after weight loss. However, in contrast with the suggestion that insulin is involved in long-term ghrelin homeostasis were findings that insulin infusions suppress (40) or have no effect (27) on ghrelin. Insulin may therefore directly or indirectly mediate the relationship between ghrelin and body weight. Alternatively, it may be both a marker of adipose tissue stores and a separate regulatory factor involved in ghrelin homeostasis.

The effects of ghrelin on energy homeostasis may be mediated by changes in energy expenditure in addition to the observed feeding effects. Conversion of total food intake to body weight was more efficient in ghrelin-treated rats compared with controls, suggesting reduced energy expenditure with ghrelin administration (41). In rodents, ghrelin administration increased RQ (42) and reduced REE (20), which may favor fat deposition, weight gain, and the development of adiposity. Conversely, after ghrelin administration in rats (42) and humans (12), no changes in total energy expenditure (42), REE, TEF, or RQ (12) were observed. We observed no relationship between any measure of total fasting or postprandial ghrelin, energy expenditure, or RQ either before or after weight loss (21). Total ghrelin was inversely related to REE and TEF in lean individuals (43), suggesting reduced energy expenditure in negative energy balance states such as during weight loss or fasting. However, Marzullo *et al.* (13) reported reduced active ghrelin levels in obese subjects with potentially impaired energy expenditure (shown by a lower REE than would be estimated from predictive equations). This decrease in energy expenditure could be an inappropriate response to the reduced ghrelin levels. The effects of ghrelin on energy expenditure may be differentially regulated in obesity, less pronounced in humans, or mediated by active ghrelin levels.

We assessed total ghrelin, which may not be the optimal measure for examining its role in acute and long-term energy homeostasis, because total and active (Ser^3 octanolyated) ghrelin are correlated in lean, but not obese, humans (13). The regulation of satiety is additionally modulated by a variety of other factors, including gastrointestinal hormones such as cholecystokinin, peptide YY, glucagon-like peptide 1, glucose-dependent inhibitory polypeptide, oxyntomodulin, peptide PP, and pancreatic polypeptide (11), some of which have been reported to be differentially regulated by macronutrient composition (44), although the literature is unclear (45). We did not measure these hormones; however, assessment of the relationship among these factors would further elucidate the relationship between dietary macronutrients and appetite.

We have confirmed the effects of obesity and weight loss on fasting and postprandial ghrelin levels in hyperinsulinemic individuals. Reductions in postprandial hunger after weight loss were predicted by improvement in fasting and postprandial ghrelin, but this accounted for only a proportion of the decrease in hunger. Decreases in surrogate markers of insulin resistance were associated with improvements in ghrelin, suggesting a regulatory role of insulin in ghrelin homeostasis. Despite a reduction in the desire to eat after an HP-LF test meal, there was no effect of macronutrient composition on changes in postprandial ghrelin before or after weight loss, and we therefore conclude that the satiating effect of dietary protein is probably mediated by factors other than ghrelin.

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